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ORIGINAL ARTICLE

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Humoral anti-idiotypic and anti-anti-idiotypic immune response in cancer patients treated with monoclonal antibody 17-1A

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Abstract A group of 96 patients with advanced colorectal carcinoma were treated with the mouse (m) or chimeric (c) (mouse variable regions × human IgG1 constant regions) monoclonal antibody (mAb) 17-1A recognizing the tumour-associated antigen GA733-2. Eighty-two of the 83 patients treated with mmAb17-1A and 69% of the patients given cmAb17-1A (n = 13) developed anti-idiotypic antibodies (ab_2). Auto-antibodies binding to tumour cells expressing GA733-2 were found in 7% of the patients. In a further 38 patients (40%) antitumour-cell antibodies, i.e. anti-anti-idiotypic antibodies (ab_3), were induced by the mAb17-1A therapy. Patients with detectable ab_3 after treatment had significantly higher ab_2 levels than those not developing ab_3. Addition of granulocyte/macrophage-colony-stimulating factor (GM-CSF) to mmAb17-1A significantly enhanced the induction of ab_2 as well as induction of anti-anti-idiotypic antibodies (ab_3), compared to mmAb17-1A alone. Patients with a high increase in antitumour-cell antibodies (ab_3) induced by the therapy lived significantly longer than patients with no or a low level of induction of ab_3 (P = 0.016). The results indicate that induction of an idiotypic network response might be an important effector mechanism in mAb therapy.

Key words Idiotypic network · Colorectal carcinoma · Monoclonal antibodies · GM-CSF.

Introduction

The clinical use of monoclonal antibodies (mAb) in cancer therapy has gained a great attention during the last decade. Therapy with mAb recognizing tumour-associated antigens (TAA) is based on the assumption that tumour cells differ from their normal counterparts in surface antigen expression. TAA may exhibit quantitative differences in comparison to the normal structure but also mutagenic and/or conformational changes [27]. Unconjugated mAb alone might induce regression of advanced disease in man [34]. Mouse mAb17-1A, raised against the TAA GA733-2 [18, 45] was used as adjuvant therapy in surgically treated patients with colorectal carcinoma (CRC) Dukes' stage C and induced a 30% increase in the survival rate at 5 years as compared to no treatment [36].

The in vivo antitumour effector functions of unconjugated mAb are not fully understood. Different direct mechanisms have been discussed: antibody-dependent cellular cytotoxicity [1], complement-dependent cytolysis [17] and apoptosis [46]. An important indirect effector function, i.e. an immunizing effect, may also be operating in vivo. In accordance with the idiotypic network theory [19], the infused antibody (ab_1) may elicit an anti-idiotypic humoral (ab_2) and T cell (T_2) response against idiotopes of ab_1. Parts of the variable regions of ab_2 might resemble the epitope that ab_1 recognizes, i.e. the nominal antigen. The structural basis for the molecular mimicry may be due to shared primary sequences or expressed at the conformational level involving either V_L or V_H segments or composit V_L and V_H determinants [4, 6, 35]. ab_2 resembling the native antigen ("internal image" anti-idiotype) may subsequently induce an anti-anti-idiotypic immune response including antibodies (ab_3) as well as T cells (T_3), which may recognize the same epitope as ab_1. It has been proposed that the idiotypic network responses, induced by mAb treatment, could be of importance for eradication of tumour cells [24].

In this report, induction of a humoral idiotypic network cascade in CRC patients treated with unconjugated mouse (m) or chimeric (c) mAb17-1A (ab_1) alone or together with granulocyte/macrophage-colony-stimulating factor (GM-CSF) is presented. The results are related to the clinical outcome of the treatment.

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Materials and methods

Therapeutic monoclonal antibodies

At the Department of Oncology (Radiumhemmet), Karolinska Hospital, clinical trials are ongoing to evaluate the antitumour effects of unconjugated mouse (m) and chimeric (c) mAb17-1A in metastatic CRC. mmAb17-1A (Centocor, Malvern, Pa., USA) is a murine IgG2A antibody raised against the TAA GA733-2 (also refered to as CO17-1A), a non-secreted 40-kDa glycoprotein, expressed by almost all human CRC tumour cells [18, 45]. cmAb17-1A (Centocor) consists of the variable regions of mmAb17-1A and the constant regions of human IgG1 [41].

Patients and treatment protocols

Patients' characteristics, treatment protocols, and clinical effects have been described elsewhere [15, 29, 32, 34]. This report includes 96 patients, 55 male and 41 female, with a median age of 61 years (range 14–81 years). All patients had metastatic disease not accessible to surgery and a Karnowsky index of at least 80% when entering the study. All tumors expressed the TAA GA733-2. Except for primary surgery, 14 patients had received chemotherapy, 5 irradiation and 1 both treatment modalities before mAb therapy. If patients progressed after mAb therapy, they were offered chemotherapy and/or irradiation.

A total of 63 patients received mmAb therapy alone (mmAb group). They were treated with single infusions (200–400 mg) of mmAb17-1A every 4–6 weeks (total dose of mmAb less than 2.0 g), either alone (n = 14), together with ex-vivo-mmAb-incubated autologous mononuclear cells (n = 15) or with a single infusion of cyclophosphamide (500 mg/m^2) preceding the mmAb infusion by 3 days (n = 13) [15, 29, 34]. A group of 21 patients received total doses of 3.6–12.0 g mmAb alone (infusions of 400–500 mg mmAb every other day or on 3 days a week) [29, 34], and 20 patients were treated with 400 mg mmAb17-1A at day 3 of a 10-day cycle of GM-CSF (250 µg/m^2 s.c. daily). The cycle was repeated at 1-month intervals for a total of four cycles (mmAb/GM-CSF group) [32]. The cmAb/GM-CSF treatment group (n = 13) received the same treatment schedule as the mmAb/GM-CSF group except that the mouse antibody was replaced by the chimeric variant.

Serum sampling

On the basis of our previous experience [9, 14] post-treatment serum samples for antibody analyses were drawn in most cases after two mAb infusions to enable detection of as many ab_3-positive patients as possible. In patients receiving mmAb treatment every other day or 3 days a week, samples were taken after completion of therapy. mmAb17-1A disappeared from serum within 7 days after an infusion [16]. To minimize the possibility that circulating mmAb17-1A interacted in the assay, all serum samples for ab_3 determinations were taken at least 2 weeks after an infusion. Owing to the longer $t_{1/2}$ of cmAb17-1A (approximately 100 h) [26] serum samples from these patients were drawn at least 4 weeks after an infusion of cmAb17-1A. It should also be added that the pharmacokinetics of repeated infusions of cmAb17-1A is stable with small variations [26].

Clinical response criteria

Clinical complete response (CR) was defined as a complete disappearance of all clinical, radiological and biochemical evidence of tumour disease. A partial response was obtained when there was a decrease of at least 50% in the product of two perpendicular diameters of all measurable disease manifestations and more than 50% decrease in the serum concentration of carcinoembryonic antigen (CEA), CA19-9 and CA50. A minor response (MR) was defined as a decrease in the product of two perpendicular diameters of at least one tumour lesion with 25%–50% and/or more than 50% decrease in the serum concentration of CEA, CA19-9 and/or CA50 and no increase (more than 25%) in any lesion. Stable disease was defined as no significant change (within 25%) in the size of all measurable lesions and no significant change (within 50%) in the serum concentration of CEA, CA19-9 and CA50 for at least 3 months. Progressive disease was defined as an increase of more than 25% in the size of at least one measurable lesion or more than 50% increase in the serum concentration of CEA, CA19-9 and/or CA50.

Determination of anti-idiotypic antibodies (ab_2)

The method for calculating the absolute concentration of ab_2 (IgG) has been described in detail elsewhere [33]. Briefly, polyclonal human IgG antibody standards with specificity for mmAb17-1A [human anti-mouse antibody (HAMA) standard] and for the Fc part of mouse IgG_{2A} (anti-isotypic IgG_{2A} antibody standard) were obtained by affinity chromatography purification of sera from mmAb17-1A-treated patients. A direct-binding enzyme-linked immunosorbent assay (ELISA) was used to determine IgG antibodies (absorbance values) against mmAb17-1A and against an irrelevant isotype-matched (IgG2A) mouse mAb [mAb425, anti-epidermal growth factor receptor (Centocor)]. The HAMA and anti-isotypic antibody standards were used to calculate the antibody concentrations against mmAb17-1A and mmAb425 respectively using the linear regression model. The absolute concentration of ab_2 was then calculated by subtracting the amount of anti-isotypic antibodies (anti-mmAb425) from that of HAMA (anti-mmAb17-1A).

Serum antibodies (1:600) against mmAb17-1A were considered to be present if the absorbance in direct ELISA exceeded 0.295 (mean + 2 SD of healthy donors, serum dilution 1:600, n = 50) [33].

Determination of tumour-cell-binding auto-antibodies and anti-anti-idiotypic antibodies (ab_3)

Serum antibodies binding to tumour cells were tested using a modified mixed haemadsorption assay [13]. The human CRC cell line, SW1116, expressing the TAA GA733-2, was treated with trypsin/EDTA (Gibco, Paisley, Scotland) for 3 min and washed twice in Leibovitz L15 complete medium (Gibco) including antibiotics (100 IE penicillin and 100 µg streptomycin/ml), glutamine (2 mM) and 10% fetal calf serum (Sera-Lab, Crawley Down, England). Viability was checked with Trypan blue. After suspension in Leibovitz L15 complete medium the target cells (8×10^4 cells/ml) (100 µl/well) were added to 96-well flat-bottomed microtitre plates (Nunc, Roskilde, Denmark) and incubated overnight at 37 °C in humidified air with 5% CO_2. The wells were then washed twice in barbital buffer (0.3 g/l $C_8H_{11}N_2NaO_3$, 0.46 g/l $C_8H_{12}N_2O_3$, 8.5 g/l NaCl, 0.168 g/l $MgCl_2$, 0.048 g/l $CaCl_2$, in distilled water, pH 7.24) containing 2% (w/v) bovine serum albumin (BSA) (Sigma, St Louis, Mo., USA) and further blocked by incubation with the same buffer (200 µl/well) for 90 min at room temperature. Heat-inactivated (56 °C for 30 min) test serum diluted 1:5 in barbital buffer with 2% BSA was added to duplicate wells (75 µl/well). The serum was incubated with SW1116 cells for 60 min at room temperature. The wells were then washed three times in barbital buffer.

An indicator system was prepared. Fresh sheep red blood cells (SRBC) were washed three times in Dulbecco's phosphate-buffered saline (PBS; Gibco) and diluted to a 0.1% cell suspension. One volume of monkey anti-SRBC antiserum (IgG; National Bacterological Laboratory, Stockholm, Sweden; 1:100 in PBS) was added to one volume of SRBC suspension and incubated for 1 h at room temperature. SRBC were then washed twice and resuspended in PBS to a concentration of 0.1%. Sheep anti-(human IgG) antiserum (National Bacteriological Laboratory) (1:100) was added to the SRBC suspension, incubated for 2 h at room temperature, washed twice and resuspended to a 0.1% solution.

A 200-µl sample of the indicator system was added to each well and incubated for 1 h at room temperature. Wells were washed three times in barbital buffer and examined in an inverted microscope. Two-hundred tumour cells were counted in each well. Cells binding three or

Table 1 Frequency of induction and concentration of anti-idiotypic IgG antibodies (ab_2) after mAb17-1A therapy of colorectal carcinoma patients in relation to treatment schedule. *mmAb* unconjugated mouse mAb, *cmAb* chimeric mAb, *GM-CSF* granulocyte/macrophage-colony-stimulating factor

Treatment group	Total no. of patients	No. of ab_2^+ patients	Ab_2 conc. geometric mean (range) (μg/ml)		
mmAb	63	62 (98%)	59.6 (0–1633)	$P < 0.001$	$P < 0.001$
mmAb/GM-CSF	20	20 (100%)	371.9 (36–948)	$P < 0.001$	
cmAb/GM-CSF	13	9 (69%)	1.6 (0–7)		

more SRBC were considered rosettes. The results are expressed as the percentage of cells forming rosettes (mean of duplicate wells).

To assess the interplate variations, barbital buffer with 2% BSA (without serum), serum from a healthy individual or a patient's serum were used as controls in all plates. In 48 consecutive plates barbital buffer with 2% BSA gave 1.2 ± 0.2% (0–6.0%) rosettes [mean ± SEM (range)] and serum from a healthy control donor 3.3 ± 0.3% (0–10.0%) rosettes. Post-treatment sera of 3 patients were also used as controls and 70.7 ± 4.3% (40.5%–99.0%) (n = 13), 55.4 ± 1.2% (40.0%–80.9%) (n = 29) and 83.4 ± 3.5% (72.9%–91.5%) (n = 6) rosettes respectively were scored. A monkey anti-(HeLa cell) serum (National Bacteriological Laboratory) was also used as a positive control and gave more than 90% rosettes. Serum from 50 healthy controls gave 1.7 ± 0.3% rosettes (mean ± SEM, range 0–11.4%). The mean + 2 SD of the results from the 50 healthy control donors was 6.0%. On the basis of these and previous results [14] serum was scored as positive for anti-tumour-cell antibodies (ab_3) if at least 15% rosettes were found. The detection limit (more than 15% rosettes) for cmAb17-1A was 0.1 μg/ml (data not shown).

Analyses for specificity of ab_3 have been published previously [9, 14]. Antibody binding to the target cells (SW1116) was inhibited by preincubation of sera with human or goat anti-mmAb17-1A idiotypic antibodies. ab_3-positive and -negative patient sera tested against tumour cell lines not expressing GA733-2 gave similar numbers of rosettes to those resulting from healthy donor sera tested against SW1116 [14]. The tumour-cell-binding capacity of ab_3^+ sera in mixed haemadsorption assay has also been confirmed by measuring the inhibition of binding of radiolabelled mmAb17-1A to GA733-2-expressing tumour cells by preincubation of the tumour cells with ab_3^+ sera [9].

To obtain an indication of the relationship between an increased serum capacity, induced by mAb therapy, to bind specifically to tumour cells (the presence of ab_3) and clinical effect a ratio was calculated. The percentage of rosettes from post-treatment serum was divided by that from the pre-treatment serum for each patient. This ratio gives information on the relative degree of ab_3 induction.

Statistics

Analyses of differences between means were determined by the Wilcoxon signed-rank test for paired and unpaired observations or by Fisher's exact test. The linear regression model was used to estimate correlation between independent observations. Survival curves were generated by the life-table method and comparisons were made by the log-rank test.

Results

Induction of anti-idiotypic (ab_2) antibodies

None of the cmAb17-1A or mmAb17-1A treated patients had detectable antibodies binding to mmAb17-1A before therapy. Of 83 patients treated with mmAb17-1A, 82 (99%) developed antibodies against mmAb17-1A (ab_1) (absorbance value against mmAb17-1A at least 0.295). The antibodies against ab_1 might bind to idiotypic as well as isotypic determinants. By comparing the absorbance values obtained against mmAb17-1A and mmAb425 (isotype-matched control antibody) respectively and by using cmAb17-1A or mmAb17-1A together with matched control antibodies in an inhibition assay all patients' sera that contained anti-mmAb17-1A antibodies were also shown to contain ab_2 [33]. Thus, 99% of the mmAb17-1A-treated patients also developed ab_2.

All patients (20/20) receiving mmAb17-1A in combination with GM-CSF mounted an ab_2 response as well. Of 13 patients treated with cmAb17-1A and GM-CSF, 9 (69%) developed detectable antibodies (ab_2) against mmAb17-1A. The difference in the frequency of ab_2 induction between the mmAb and cmAb treatment regimens was statistically significant ($P < 0.02$). The absolute concentrations of ab_2 varied markedly between the different treatment groups (Table 1).

Induction of anti-anti-idiotypic antibodies (ab_3)

Seven (6 patients treated with mmAb and 1 patient with cmAb) of the 96 patients (7%) had antibodies binding to human CRC cells before treatment. After therapy 15 out of 20 patients (75%) in the mmAb/GM-CSF group had ab_3 while only 23 of 63 patients (37%) treated with mmAb alone were positive ($P < 0.005$).

When evaluating human ab_3 by mixed haemadsorption assay in patients treated with cmAb17-1A care has to be taken because of the long plasma half-life of cmAb17-1A (see Materials and methods). In this patient group not only were more than 15% rosettes required but also a significant increase in the percentage of rosettes when values on day 1 of treatment cycles III or IV were compared with that of cycle II. With this strict criterion 6 patients (46%) in the cmAb/GM-CSF group were considered to show induction of ab_3 even though anti-tumour-cell antibodies were found after treatment in a further 2 patients (patients 2 and 9) (Table 2). Those 6 patients who developed ab_3 also had induction of ab_2. The kinetics of the ab_3 response in cmAb17-1A-treated patients is exemplified in Fig. 1. The frequency of post-treatment ab_3^+ patients in the cmAb/GM-

Table 2 Auto-antibodies and induced anti-anti-idiotypic antibodies (ab_3) binding to tumour cells (percentage forming rosettes) in patients treated with chimeric mAb17-1A at the start of the treatment cycles. Tumour-cell-binding antibodies were determined by mixed haemoadsorption assay. Serum was scored as positive if more than 15% rosettes were counted. Post-treatment samples also had to have a significant increase in the percentage of rosettes at the start of cycle III or IV as compared to cycle II. The first positive value in each patient is in bold type. *ND* = not done

Patient	Cycle I	Cycle II	Cycle III	Cycle IV
1	11	11	**28**	ND
3	10	5	12	**24**
5	11	18	16	**26**
6	13	29	41	**56**
7	11	10	**40**	48
8	6	12	9	**19**
13	**47**	62	40	39
2	12	37	21	23
9	11	20	19	18
4	9	14	15	ND
10	6	9	8	2
11	6	12	7	5
12	6	12	7	9

CSF group (7/13; 54%) was statistically not different from that of the mmAb groups.

Relationship between anti-idiotypic and anti-anti-idiotypic antibodies

There was a statistically significant correlation between the ab_2 concentration and the percentage of ab_3 rosettes in post-treatment sera of all patients ($r = 0.347$; $P < 0.001$; $n = 96$), which was even stronger for patients treated with mmAb alone ($r = 0.480$; $P < 0.001$; $n = 63$). Accordingly, sera of post-treatment ab_3^+ patients ($n = 45$) also had significantly higher ab_2 concentrations than those of ab_3^- patients ($n = 51$): 202.5 μg/ml (range 1.1 – 1633.6 μg/ml) compared to 38.6 μg/ml (range 0 – 1120.5 μg/ml) ($P = 0.005$).

The time kinetics for induction of ab_2 and ab_3 are exemplified in Fig. 2.

Table 3 Relationship between rosette indices and median survival time in mAb17-1A-treated colorectal carcinoma patients

Rosette index cut-off level	Rosette index below cut-off		Rosette index above cut-off		*P*
	No. of patients	Survival (weeks)	No. of patients	Survival (weeks)	
1.0	23	37.4	73	47.1	0.104
1.6	48	43.9	48	54.6	0.079
2.0	55	42.4	41	58.1	0.032
3.0	74	45.7	22	73.1	0.132

Relationship between ab_2, ab_3 and clinical effects

The concentration of ab_2 has previously been shown to correlate with the appearance of immediate-type allergic reactions (type I). There also seemed to be a relation to tumour response (complete, partial, minor or stable disease) and survival, i.e. a tumour response was only seen in patients with an ab_2 concentration at least 15 μg/ml. The median survival for patients with ab_2 concentrations of at least 15 μg/ml ($n = 65$) was 15 months as opposed to 9 months for patients ($n = 21$) with a lower ab_2 concentration ($P = 0.01$) [33].

When analysing a relationship between clinical effects and ab_3 a rosette index (see Materials and methods) was used. An index above 1.0 indicates an increase in the number of rosette-forming tumour cells after therapy as compared to the pretreatment value, i.e. an augmented serum level of antibodies binding to tumour cells. The median post-treatment rosette index for all patients was 1.61 (range 0.19 – 16.7). To evaluate a relation between the

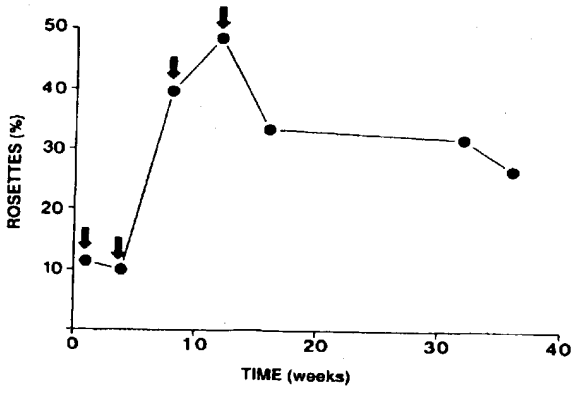

Fig. 1 Kinetics of tumour-cell-binding antibodies (ab_3) measured by mixed haemadsorption assay in patient 7 treated with chimeric mAb17-1A. *Arrows* infusions of 400 mg cmAb17-1A

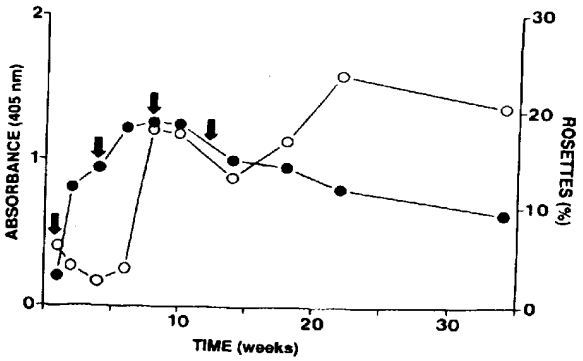

Fig. 2 Kinetics of induction of ab_2 (●) and ab_3 (○) in patient 14 treated with mmAb17-1A in combination with granulocyte/macrophage-colony-stimulating factor. *Arrows* infusions of mmAb

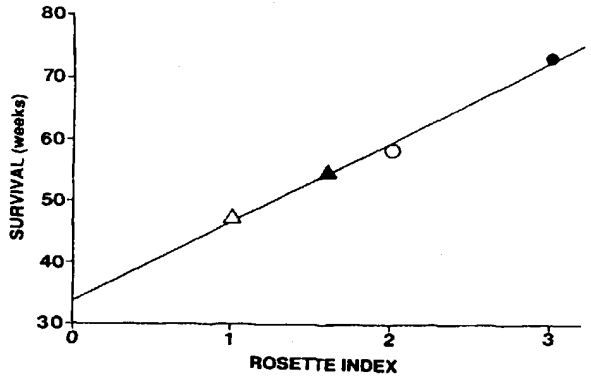

Fig. 3 Relationship between rosette index and median survival time of all mAb17-1A-treated patients with an index value above the indicated ratio ($r = 0.9961$; $P < 0.005$; $n = 4$). Number of patients for each point (see Table 3): △ $n = 73$; ▲ $n = 48$; ○ $n = 41$; ● $n = 22$

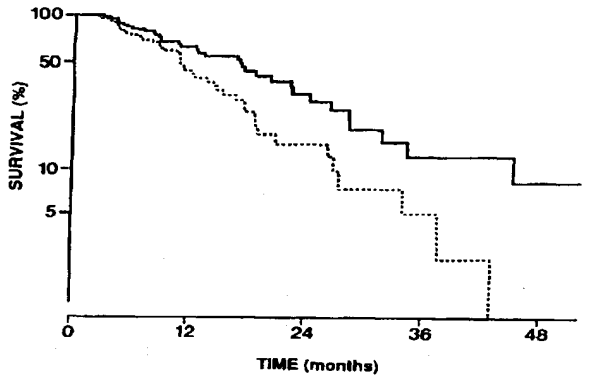

Fig. 4 Survival of mAb17-1A-treated patients with a rosette index above 2.0 (——) ($n = 41$) and below 2.0 (- - - -) ($n = 55$). The difference was statistically significant ($P < 0.016$)

rosette index and survival, different cut-off levels were analyzed (Table 3). There was a highly statistically significant correlation between the median survival time and the rosette index ($r = 0.9961$, $P < 0.005$), i.e. the higher the index the longer the survival (Fig. 3). Survival of all patients with a rosette index above 2.0 was significantly better than for patients with an index below this value ($P = 0.016$) (Fig. 4). There was no statistically significant difference between the two groups with regard to age, site of metastases, degree of tumour differentiation, tumour stage at primary surgery (Dukes' classification), Karnofsky index and median time from surgery to the start of therapy (data not shown).

The relationship between clinical response to mAb17-1A treatment and the rosette index was also analysed. The median rosette index for responding patients was 2.43 (range 0.20–16.67) and 1.49 (range 0.22–15.72) for non-responding patients. Of 45 post-treatment ab_3^+ patients, 11 (24%) achieved a tumour response while only 6 of 51 ab_3^- patients (12%) responded ($P = 0.08$).

Discussion

The in vivo antitumour effect of unconjugated mAb (ab_1) might not only be dependent on direct cytotoxic mechanisms, such as antibody-dependent cellular cytotoxicity, complement-dependent cytolysis and apoptosis, but also be due to the induction of an immune reaction against ab_1. The mounting of an idiotypic network response [19] may be an important secondary effector function. Induction of ab_2 fulfilling functional criteria for resembling the nominal tumour antigen in patients exposed to ab_1 has been proven, i.e. blocking of binding of ab_1 to the native antigen and induction of antibodies with ab_1-like binding specificity on immunization across species barriers [2, 39, 43, 44]. Such ab_2 might have structural homology with the nominal antigen. Immunization of CRC patients with human monoclonal ab_2 showed the induction of ab_3 binding to the tumour antigen as well as to ab_2 [12]. Immunization with human ab_2 could also give rise to a T cell immunity, demonstrated in vivo by the delayed-type hypersensitivity reaction and in vitro by T cells recognizing the isolated TAA and TAA-expressing tumour cells [8, 12, 37]. Furthermore in ab_1-treated patients the induction of ab_3 binding to TAA-expressing tumour cells has been shown [9, 10, 14, 47]. The ab_3 response correlated favourably to survival [5, 14].

The aim of the present study was to analyse the induction of ab_2 and ab_3 in patients with metastatic CRC who had been treated with mouse or chimeric mAb17-1A. ab_2 was found in 99% of mmAb-treated and 69% of cmAb-treated patients. In other studies, the frequencies of anti-ab_1 induction varied between 54% and 93% for mmAb [7, 25, 39, 40, 42] and 10%–75% for cmAb-treated [3, 22, 26] patients. The variation in antibody induction might be related to differences in treatment schedules, sampling times, antibody detection techniques and most probably in the patient materials. The significantly higher levels of ab_2 found in mmAb/GM-CSF-treated patients as compared to the other treatment groups were most likely due to the stimulating effect of GM-CSF on antigen-presenting cells [30]. The low concentration of ab_2 in cmAb/GM-CSF-treated patients might depend on the chimerization of the antibody molecule, i.e. fewer foreign epitopes are present to serve as T helper epitopes as compared to mmAb.

Analyses of ab_3 induction after ab_1 therapy have not previously been published for a large patient population. Overall 7 of 96 patients (7%) had anti-tumour-cell antibodies before treatment (auto-antibodies) and a further 38 patients (40%) showed induction of ab_3. A significant correlation between post-treatment ab_2 and ab_3 values was found, which might be expected if ab_2 were to induce an ab_3 response.

The potential clinical benefit of ab_3 induction, as previously proposed [5, 14], was also analysed. The increase in

serum antibody-binding activity to tumour cells (rosette index) during treatment was significantly related to survival. This might indicate that ab_3 induction may be of clinical benefit. In line with this suggestion is the observation that naturally occurring anti-melanoma antibodies correlated positively to survival [20]. However, induction of anti-tumour T cells through the idiotypic network might be an even more important idiotypic network effector function. The induction of T cells specifically recognizing ab_2 and the nominal TAA has been shown to correlate with major tumour response [9, 11, 28]. These results are in agreement with animal data indicating that tumour-specific T cells are most important for tumour rejection as compared to antibodies [21, 23, 31].

In conclusion, the present study showed a relation between the induction of ab_2 and ab_3 in patients treated with monoclonal antibodies. The addition of GM-CSF increased the serum concentration of ab_2 as well as the frequency of ab_3 induction. Further clinical benefit of an idiotypic network response following mAb therapy was suggested by a relation between ab_3 response and survival. To improve mAb therapy it should be of the utmost importance to have a detailed knowledge of mechanisms participating in tumour cell control in vivo to be able to modulate favourably host immune functions involved in antitumour effects mediated by monoclonal antibodies.

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